Use of a personal computer to determine the statistical validity of antibody identification by Fisher's exact method

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Conclusive identification of irregular red blood cell (RBC) antibodies requires that the serum be tested against sufficient numbers of antigen-positive and antigen-negative RBC samples. This ensures that the observed reactivity pattern is not due to chance. The probability (p) that panel results would be obtained by chance is calculated by use of Fisher's exact method for the analysis of 2 x 2 contingency tables.[1-4]

The table is constucted as follows:

	Red Cells		
Serum reactions	Antigen present	Antigen absent	Total
Positive	A	B	A + B
Negative	C	D	C + D
	A + C	B + D	N

A = number of positive reactions observed with antigen-positive RBCs

B = number of positive reactions observed with antigen-negative RBCs

C = number of negative reactions observed with antigen-positive RBCs

D = number of negative reactions observed with antigen-negative RBCs

N = total number of RBC samples tested

The probability (p) is calculated as follows:

$$\frac{(A + B)!\ X\ (C + D)!\ X\ (A + C)!\ +\ (B + D)!}{N!\ X\ A!\ X\ B!\ X\ C!\ X\ D!}$$

The American Association of Blood Banks[3] and the Canadian Red Cross[4] consider a p of 0.05 (5%) or less an acceptable value for panel interpretations to be considered statistically valid. Current standards for American Red Cross Blood Services Reference Laboratories[5] require identification at a significance level of 0.01 (1%). Regardless of the standard used, if p exceeds the minimum acceptable value, additional serologic testing is required to bring p below this level before the interpretation can be accepted.

The numerous individual calculations required make the calculation of p a time consuming affair, even with the aid of a hand calculator. For this reason, many blood banks do not calulate p values for their panel interpretations, relying on faith that commercial panels have sufficient numbers of antigen-positive and antigen-negative RBCs to permit statistically valid interpretations for all panels run. This presentation describes two programs designed for a personal computer (PC) that can be used to rapidly calculate p values and assess the validity of panel interpretations.

Computer Programs

Two PC programs have been developed that calculate p based on panel results and give advice on additional serologic testing when p is outside the acceptable limits. The first uses a p value of 0.05 as the acceptable value for testing validity. The second uses a p value of 0.01. Aside from this difference, the programs are the same. The programs were written in Microsoft

BASIC*-80, Rev. 5.21., for PCs with an 80-column monitor display, but they can be easily modified for PCs that use a different form of BASIC and/or use a different size display. The use of a printer is not required, but the programs can be modified to permit a printout of the results.

When either program is run, the user is given the option, before any calculations are attempted, of viewing instructions on the construction of 2 x 2 contigency tables, the calculation of p, and use of the program itself. Once the instructions have been viewed or the option bypassed, the programs proceed to data entry and the calculation of p.

The programs then instruct the user to enter the following data, in this order:

1. The identity of the antibody, based on panel interpretation
2. The total number of RBC samples of known antigenic makeup tested against the serum (N)
3. The number of antigen-positive cells that react with the serum (A)
4. The number of antigen-negative cells that react with the serum (B)
5. The number of antigen-positive cells that do not react with the serum (C)
6. The number of antigen-negative cells that do not react with the serum (D).

Following the last data entry, a diagnostic check is made to ensure against data entry errors, such as the inclusion of cord cells or the autocontrol in the total number of samples tested entry. If an entry error is detected, the user is informed and instructed to recheck and reenter the data.

When it has been established that the data have been properly entered, the selected program then calculates p, and the result is displayed. The time from final data entry to the display of the calculated p is approximately one second. If the calculated value is equal to or less than the established acceptable value, the user is informed that this p is statistically significant, that is, small enough for the identification to be considered statistically valid. If the calculated value is greater than the acceptable value, the user is informed that p is not statistically significant, the interpretation is not valid, and that additional serologic testing is required.

The programs then proceed to a series of data analysis subroutines that analyze the data entered and provide the user with recommendations for further testing that will reduce p to an acceptable level. After viewing the recommendations, the user is asked whether or not additional calculations are to be made. If the response is "no," the program is terminated. If the response is "yes," the programs return to the data entry section to begin the next calculation.

Examples of the program for determination of the statistical validity of antibody identification by Fisher's exact method using $p \leq 0.01$ as the acceptable level for testing validity follow.

Example 1: *A calculation of p.*

Based on your interpretation of panel results, what antibody appears to be in the patient's serum?
anti-? C ⟨RETURN⟩

Enter the total number of panel cells run against this serum. (DO NOT include cord cells or the autocontrol.)
? 10 ⟨RETURN⟩

Enter the number of C-positive cells that demonstrate a positive reaction with this serum.
? 3 ⟨RETURN⟩

Enter the number of C-positive cells that demonstrate a negative reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of C-negative cells that demonstrate a positive reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of C-negative cells that demonstrate a negative reaction with this serum.
? 7 ⟨RETURN⟩

The calculated p value for this panel interpretation is 0.00833

This p value is small enough to make the identification of anti-C statistically valid.

Do you want to calculate another p value (Y/N)?
Y ⟨RETURN⟩

Example 2: *Demonstration of the error trap detecting inclusion of cord cell and autocontrol results, followed by correct data entry.*

Based on your interpretation of panel results, what antibody appears to be in the patient's serum?

*Microsoft BASIC is a trademark of Microsoft, Inc., Odessa, TX.

anti-? Fya ⟨RETURN⟩

Enter the total number of panel cells run against this serum. (DO NOT include cord cells or the autocontrol.)
? 12 ⟨RETURN⟩

Enter the number of Fya-positive cells that demonstrate a positive reaction with this serum.
? 6 ⟨RETURN⟩

Enter the number of Fya-positive cells that demonstrate a negative reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of Fya-negative cells that demonstrate a positive reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of Fya-negative cells that demonstrate a negative reaction with this serum.
? 4 ⟨RETURN⟩

The values you have entered do not add up correctly. Check your data to make sure that:
1. You understand the data categories correctly.
and/or
2. You are not including cord cells and/or the autocontrol in your total.

Press RETURN when you are ready to continue.
? ⟨RETURN⟩

Enter the total number of panel cells run against this serum. (DO NOT include cord cells or the autocontrol.)
? 10 ⟨RETURN⟩

Enter the number of Fya-positive cells that demonstrate a positive reaction with this serum.
? 6 ⟨RETURN⟩

Enter the number of Fya-positive cells that demonstrate a negative reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of Fya-negative cells that demonstrate a positive reaction with this serum.
? 0 ⟨RETURN⟩
Enter the number of Fya-negative cells that demonstrate a negative reaction with this serum.
? 4 ⟨RETURN⟩

The calculated p value for this panel interpretation is 0.00476.

This p value is small enough to make the identification of anti-Fya statistically valid.

Do you want to calculate another p value (Y/N)?
Y ⟨RETURN⟩

Example 3: *Demonstration of data analysis subroutines when calculated p value exceeds the acceptable limit.*

Based on your interpretation of panel results, what antibody appears to be in the patient's serum?
anti? k ⟨RETURN⟩

Enter the total number of panel cells run against this serum. (DO NOT include cord cells or the autocontrol.)
? 10 ⟨RETURN⟩

Enter the number of k-positive cells that demonstrate a positive reaction with this serum.
? 9 ⟨RETURN⟩

Enter the number of k-positive cells that demonstrate a negative reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of k-negative cells that demonstrate a positive reaction with this serum.
? 0 ⟨RETURN⟩

Enter the number of k-negative cells that demonstrate a negative reaction with this serum.
? 1 ⟨RETURN⟩

The calculated p value for this panel interpretation is 0.1.

This p value is not statistically significant and the identification of anti-k is not valid.

Press RETURN to view the recommendation(s) for additional testing to reduce p below 0.01.

1. Increase the number of k-negative cells tested to at least 3.

Press RETURN when you are ready to continue.
? ⟨RETURN⟩

Do you want to calculate another p value (Y/N)?
N ⟨RETURN⟩

Discussion

The goal of developing these programs was to create user-friendly software that would rapidly calculate p values and provide advice for additional testing in

those instances when p exceeded the minimum acceptable level. In designing the programs, several panels were analyzed, using hypothetical examples of reactivity patterns, to determine what conditions would result in a p value exceeding the minimum acceptable level. Five such patterns were found using the 0.05 level:

1. Failure to run at least ten cells in a panel
2. Failure to run at least two cells which are antigen-positive for the suspected antibody
3. Failure to run at least two cells that are antigen-negative for the suspected antibody
4. Negative reaction(s) by one or more cells that are antigen-positive
5. Positive reaction(s) by one or more cells that are antigen-negative.

These patterns were similar for p at the 0.01 level with the exception that, for patterns listed as 2 and 3 above, failure to include at least three antigen-positive cells and three antigen-negative cells, respectively, would result in a p value exceeding 0.01.

Based on these findings, five diagnostic subroutines were developed that would analyze each of the numerical data entries when an unacceptable p value was found. These subroutines determine which of the above conditions were involved and then make specific recommendations for additional serologic testing.

In a review of several lots of commercial reagent red cell panels composed of 10, 11, or 16 red cell samples, it was noted that nearly all 10- and 11-cell panels and many 16-cell panels contained an insufficient number of RBCs positive for low-incidence antigens (C^w, V, VS, Kp^a, Js^a, Lu^a) or negative for high-incidence antigens (e, k, Kp^b, Js^b, Lu^b) to permit valid identification of the corresponding antibodies at a p value ≤ 0.05 and none permitted valid identification at a p value ≤ 0.01. Most lots of 10- or 11-cell panels permitted identification of anti-E, -c, -e, and -K with a p ≤ 0.05; few of these lots permit identification with a p ≤ 0.01 because they do not contain sufficient E+, K+, c- or e- RBCs. Occasionally, the makeup of certain lots of 10- or 11-cell panels would not permit statistically valid identification at either acceptable p level because they contained an inappropriate ratio of RBCs with homozygous and heterozygous expression of an antigen. These findings underscore the need to calculate p values for all panel results in order to ensure that proper interpretations are being made.

Conclusions

These two programs allow PC users to rapidly calculate p values to determine the validity of panel interpretations. The diagnostic subroutines incorporated in them also provide specific recommendations for additional testing when p exceeds the selected acceptable level.

References

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Author's note: Programs 1 and 2 may be obtained in their entirety from the author by request.